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Mechanism of Hemolysis Caused by Ultrasonic Irradiation. I

W. ROMANOWSKI, A. FELTYNOWSKI, and J. LITWIN

*Human Physiology Department of the Academy of Medicine, Warsaw,
and State Institute of Hygiene, Warsaw*

The authors investigated the mechanism of hemolysis caused by the ultrasonic irradiation in several experiments under special conditions, and they have obtained some interesting results.

The blood of the dog treated with heparine and next diluted three times by Tyrode's liquid was irradiated for 15 min. from a generator of the power of 3 W/cm² and at the frequency of 800 Kc. Then the specimen was formed from the erythrocytes irradiated and from the controls. The specimens were shadowcast with chromium and were observed under the Siegbahn Schoenander electron microscope.

All the blood cells from the irradiated specimen were much smaller than the controls and attained 1/3 of their previous size. The blood cells had no characteristic "delta" form in the centre, and their shadows were longer than those of the controls. This indicated that the erythrocytes transformed into a spherical form.

We find that some results of Sibuya (2) and those of Lindemann (1) though contrary to their interpretation, give evidence that the spherical form is

one of the forms in which the erythrocytes change when subjected to ultrasonic treatment, and this change may precede the hemolysis. It is now difficult to decide whether it is caused by the denaturation by the ultrasonic radiation of a characteristic protein in the erythrocyte membrane which gives shape "biscuit", or by the change in pH or temperature or by other factors.

When irradiating with a smaller dose, changes similar to those described by Lindemann were observed, i.e. convex and concave deformations on the surface of the blood cell. The appearance of these forms can be explained by a non-uniform denaturation of the protein over the whole surface.

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The Mechanism of Hemolysis Caused by Ultrasonic Irradiation. II

W. ROMANOWSKI and A. FELTYNOWSKI

*Human Physiology Department of the Academy of Medicine, Warsaw,
and State Institute of Hygiene, Warsaw*

By means of an ultrasonic 3 W/cm² generator at the frequency of 800 Kc the authors irradiated mice in a special vessel submerged into another one filled with oil (cooled with ice and water). The blood specimens were taken from the tail and then investigated under the electron microscope. The irradiation times were 9-13 min. (mortal dosage) and 5 min.

After irradiating with a mortal dosage nearly all the erythrocytes were greatly changed. Their sizes were much smaller than those of the control ones, and their shapes were also changed. Some

smaller or greater convex and concave deformations were observed. After five minutes' irradiation such changes have also been observed, but not on all blood cells.

These deformations are thought to be caused by a non-uniform denaturation of the protein on the surface, which gives a "biscuit" form to the erythrocytes.

Similar changes in size and shape of the erythrocytes were obtained in *in vitro* experiments (preceding communication).

Filamentous Forms of Influenza Viruses

^{in form}
A. FELTYNOWSKI

State Institute of Hygiene, Department of Virology, Warsaw

FILAMENTOUS forms of influenza viruses were first observed by Mosley & Wyckoff (8). Chu, Dawson & Elford (3) found that recently-isolated strains of the A-type frequently showed filamentous forms. They demonstrated that these forms were characterized by several properties of the elementary bodies of the influenza virus. Hoyle (7) found in the dark field of the light microscope protrusions in the infected allantois membrane and concluded that they contain virus material. It was Wyckoff (11) who first examined the membrane of the chicken embryo infected

by influenza virus on thin sections and found that the cells apparently excreted influenza virus filaments.

In a review of Angulo in 1951, the argument was presented that the filamentous forms were no viruses but breakdown products of the cell cytoplasm. In a previous paper (6) this argument was proven to be non-valid.

Most investigators concerned with the morphology of the influenza virus have described either the elementary round bodies or the elongated forms from

isol through short rods to filaments Archetti (2) and Draganow (3) who give a complicated description of the influenza virus, called by Draganow N.W.-bodies.

In this paper we are concerned with the observations on the influenza virus strains isolated in Poland in the Virology Department of the State Institute of Hygiene headed by Prof. F. Przemycki during the influenza epidemics in the years 1953, 1954 and 1955.

The virus strains were as follows:—

The 1953 epidemic: 23 strains were isolated, from which 14 were examined under the electron microscope. The isolated strains belonged to the A-type and only 2 were established as belonging to A-prime-type (see (9) where all methods are given).

The 1954 epidemic: 12 strains were isolated, all examined morphologically and found to belong to the A-prime-type.

The 1955 epidemic: 6 strains were isolated, all belonging to B-type. The laboratory characteristics of the last two epidemics are given in (10).

The preparation of the specimen for the electron microscope is described in the paper previously mentioned (6).

One strain isolated from the 1954 epidemic (so-called "LAK"), belonging to the A-prime type was examined morphologically in more detail. Twenty passages were performed and after each of them the strain was observed under the electron microscope. The optimal passages were 10^{-1} or 5×10^{-2} .

The main result was that the isolated strains were different morphologically. Some of them were filamentous, other had round forms.

The well-known strain PR8 (6) showed long and filamentous forms but it ought to be considered as a strain of round forms because the elementary round bodies are prevalent here. One of the strains isolated in 1953 showed exceptional concentration of long filaments. This strain was a mixed one (obtained from several washings), and it may be interesting to note that the individual strains did not show any or very little filamentous forms. The filaments have thus become prevalent in the mixed strain. Another strain of the same epidemic (so-called "Z.Z."), belonging to the A-prime type, can also be considered as "filamentous".

Most of the strains from this epidemic belonged to the A-type and they are of round forms. The long filamentous forms were not present, but the characteristic short beaded forms consisting of 3-4 beads were also observed (fig. 1).

All the 12 strains from the 1954 epidemic belonged to the A-prime type and all should be considered as filamentous. They showed a great variety of forms (fig. 2).

After several passages of the "LAK" strain the concentration of the filaments decreased.

All the strains isolated in the year 1955 belong to the B-type. They consisted of elementary round bodies only.

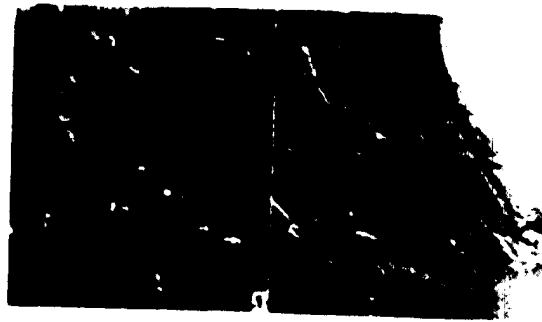


Fig. 1. Strain K.M. Type A-1953. Magnification

Fig. 2. Strain TM.1. Type A'-1954. Magnification

In the "filamentous" strains the hemagglutination titer of the infected allantois fluid was very low and amounted from 1:40 up to 1:160. Although the morphological pattern of the "LAK" strain after the 1st and 15th passages was different, no difference was established in the antigenic structure.

The "filamentous" strains of the virus show strong polymorphism. In addition to the round bodies, there are also "transitory" forms (from spheres to filaments) and complicated filamentous forms which were called by Draganow N.W.-forms. These polymorphic bodies are characteristic only for the recently-isolated strains and were observed on the A-prime-type. All the B-type strains turned out to be those of round forms.

In my view the filamentous forms present an earlier stage of the development of the virus which then pass into elementary round bodies.

The present work was carried out under the guidance of Professor Przemycki to whom I express my thanks. I should like to acknowledge the assistance of Mrs. K. Zgorzelska and Dr. B. Malczewski in the preparation of the virological samples.

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WYKAZ PRAC

O PROBLEMATYCE BIOLOGICZNEJ
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